

Protective effect of butin against ischemia/reperfusion-induced myocardial injury in diabetic mice: involvement of the AMPK/GSK-3 β /Nrf2 signaling pathway

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Supplemental figures

Supplementary table 1: Effect of BUT on the levels of blood glucose.

Parameter	Control	Model	BUT(10)	BUT(20)	BUT(40)	MET
Blood glucose (mM)	7.7±2.4	22.4±3.2 ^{##}	20.3±2.8	16.3±3.3 ^{**}	13.6±3.1 ^{**}	13.1±2.7 ^{**}

Values (n=6–8 per group) are expressed as means ± SD. ^{##}P<0.01 vs control group, ^{**}P<0.01 vs model group. Control group: normal mice; Model: diabetic mice; BUT(10), BUT(20), BUT(40): diabetic mice were treated with 10, 20 and 40 mg/kg BUT, respectively; MET: diabetic mice were treated with 50 mg/kg MET.

Supplementary table 2: The relationship between the size of AAR and the size of Infarct was plotted and analyzed using analysis of covariance.

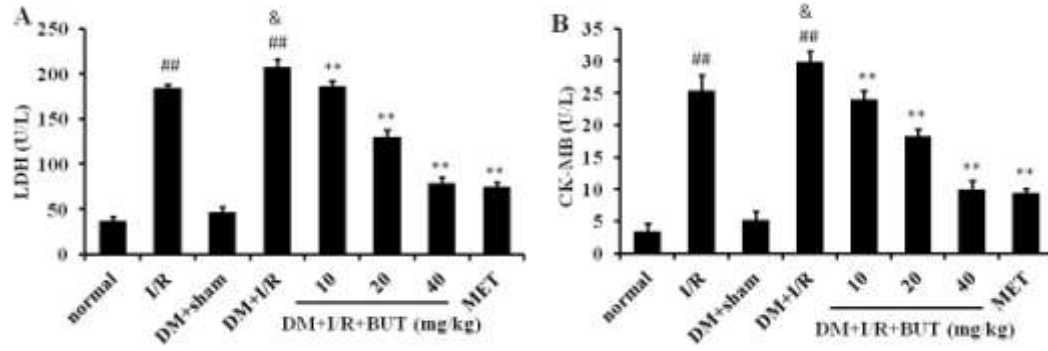
Tests of Between-Subjects Effects

Dependent Variable: Infarct

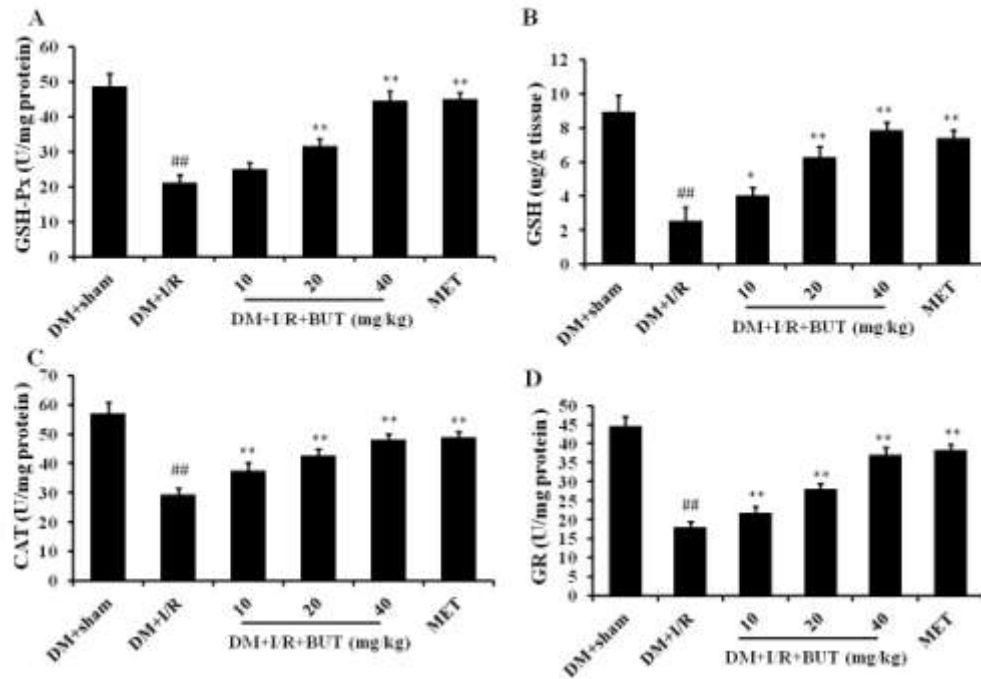
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2662.247 ^a	8	332.781	70.258	.000
Intercept	.026	1	.026	.005	.942
AAR	13.605	1	13.605	2.872	.111
GROUP	1253.281	7	179.040	37.800	.000
Error	71.048	15	4.737		
Total	6031.069	24			
Corrected Total	2733.295	23			

a. R Squared = .974 (Adjusted R Squared = .960)

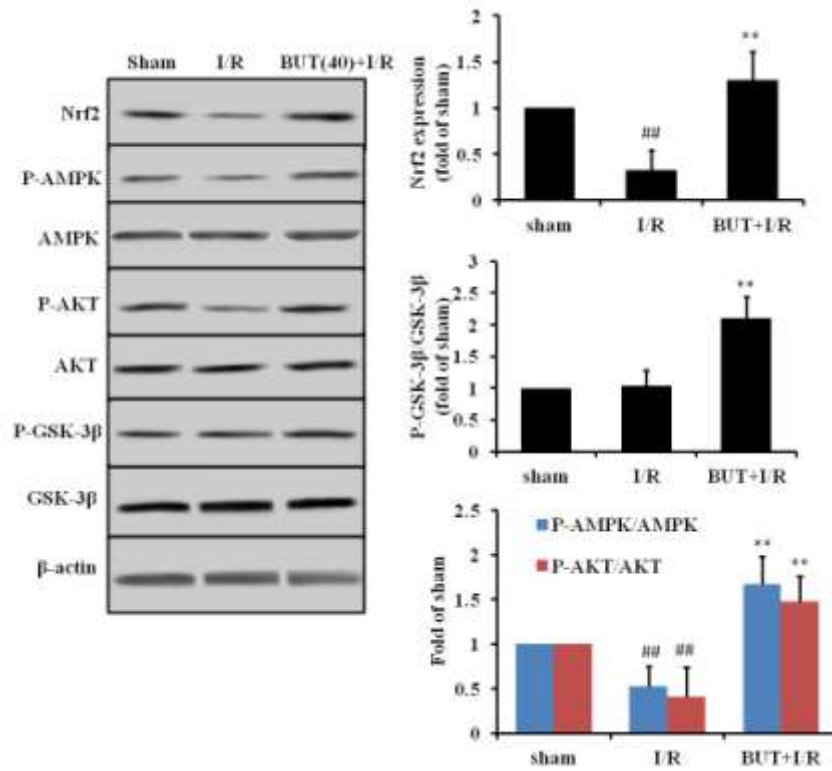
Supplementary figure 1: (A) Plasma creatine kinase-MB (CK-MB) and (B) lactate dehydrogenase (LDH) levels. Values (n=6–8 per group) are expressed as means \pm SD. $^{##}P<0.01$ vs DM+sham group, $^{**}P<0.01$ vs DM+I/R group, $^{\&}P<0.05$ vs I/R group.



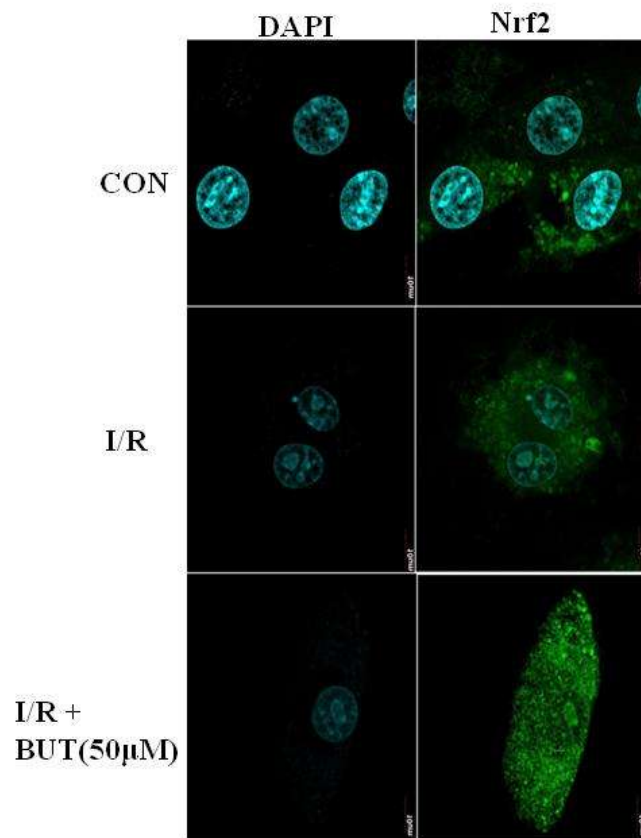
Supplemental figure 2: BUT up-regulated the expression of GSH-Px, GSH, CAT, GR in the heart. Mice received I/R treatment in the presence or absence of BUT pre-treatment, the expression level of GSH-Px (A), GSH (B), CAT (C) and GR (D) in the heart were measured as described in Materials and methods. Values (n=6–8 per group) are expressed as means \pm SD. $^{##}P<0.01$ vs DM+sham group, $^{**}P<0.01$ vs DM+I/R group.



Supplemental figure 3: Effects of BUT on AMPK, Akt and GSK3 β phosphorylation and Nrf2 expression in heart treated with I/R. Immunoblotting of protein extracts from heart of mice treated with BUT (40 mg/kg). Levels of Nrf2 expression and AMPK, Akt, GSK3 β phosphorylation in the hearts of mice treated with BUT. The columns and errors bars represent means \pm SD. ^{###} P <0.01 vs sham group, ^{**} P <0.01 vs I/R group.



Supplemental figure 4: BUT induced the cytoplasm expression and nuclear translocation of Nrf2 determined by immunofluorescence staining. Left panel: green fluorescence showing Nrf2 localization; Middle panel: stained nucleus with DAPI.



Supplemental figure 5: Cell viability (A) and Ros levels (B) were determined after siRNA transfection. The columns and errors bars represent means \pm SD. ** $P < 0.01$ vs I/R group. ## $P < 0.01$ vs control group. && $P < 0.01$ and \$\$ $P < 0.01$ vs scrambled control RNA.

